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Correlation of rotational thromboelastometry (ROTEM) parameters with platelet count and their ability to predict thrombocytopenia in dogs

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Ziel der Studie war es die Korrelation der Plättchenzahl (PLT) mit Parametern der Rotationsthromboelastometrie (ROTEM) und ROTEM Cut-off-Werte zur Identifikation von Thrombozytopenie bei Hunden zu beschreiben.
Krankengeschichten von 113 Hunden mit parallelen EXTEM (ROTEM mit Gewebefaktor aktiviert), FIBTEM (EXTEM mit Zytochalasin D) und PLT Werten wurden retrospektiv ausgewertet. Signalement, Behandlungen vor Blutanalyse, Hämatokrit, EXTEM/FIBTEM maximale Gerinnselstärke (MCF_{EXTEM}, MCF_{FIBTEM}) und -elastizität (MCE_{EXTEM}, MCE_{FIBTEM}) und EXTEM maximale Lyse (ML_{EXTEM}) wurden den Krankengeschichten und der Datenbank entnommen. Delta (Δ) MCF wurde als MCF_{EXTEM} - MCF_{FIBTEM} und ΔMCE als MCE_{EXTEM} – MCE_{FIBTEM} berechnet. Die PLT wurden mit Spearman-Rho Analyse zu MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF und ΔMCE korreliert. Um die Voraussagekraft des ROTEMs für Thrombozytopenie zu bestimmen wurden Receiver Operating Characteristics durchgeführt. MCF_{EXTEM} <49mm, MCE_{EXTEM} <93, ΔMCF <42mm und ΔMCE <90 sagten eine Thrombozytopenie <60 x 10^9/L mit einer Sensitivität von 90% und einer Spezifität von 78% voraus, mit einem negativ prädiktiven Wert von >97% für alle 4 Parameter.
PLT korrelierten moderat aber signifikant mit allen evaluierten ROTEM Parametern. Alle Parameter konnten eine Thrombozytopenie <60 x 10^9/L voraussagen mit einem hohen negativ prädiktiven Wert, während die Sensitivität eine Thrombozytopenie vorauszusagen moderat und der positiv prädiktive Wert tief war.

Hund, EXTEM, FIBTEM, ROTEM, Plättchenkomponente

Vetsuisse Faculty University of Zurich (2019)

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Correlation of rotational thromboelastometry (ROTEM) parameters with platelet count and their ability to predict thrombocytopenia in dogs

The study objectives were to evaluate the correlation between platelet count (PLT) and rotational thromboelastometry (ROTEM) parameters and to determine ROTEM cut-off values for identification of thrombocytopenia in dogs.
Medical records of 113 dogs with concurrent EXTEM (ROTEM activated by tissue factor), FIBTEM (EXTEM with cytochalasin D) analysis and PLT were retrospectively reviewed. Signalment, treatment prior to analysis, hematocrit, EXTEM/FIBTEM maximum clot firmness (MCF_{EXTEM}, MCF_{FIBTEM}), EXTEM/FIBTEM maximum clot elasticity (MCE_{EXTEM}, MCE_{FIBTEM}) and EXTEM maximum lysis (ML_{EXTEM}) were extracted from patient records and ROTEM database. Delta (Δ) MCF was calculated as MCF_{EXTEM} - MCF_{FIBTEM} and ΔMCE as MCE_{EXTEM} – MCE_{FIBTEM}. The PLT was correlated to MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE using Spearman-Rho analysis.
The ability to predict thrombocytopenia was evaluated with receiver operating characteristics (ROC). MCF_{EXTEM} <49mm, MCE_{EXTEM} <93, ΔMCF <42mm and ΔMCE <90 predicted thrombocytopenia <60 x 10^9/L with a sensitivity of 90% and a specificity of 78% with a negative predictive value >97% for all 4 parameters.
PLT in dogs correlated moderately but significantly with all evaluated ROTEM parameters. All parameters were able to rule out thrombocytopenia <60 x 10^9/L with a high negative predictive value, while the sensitivity to predict thrombocytopenia was only moderate and the positive predictive value was low.

Canine, EXTEM, FIBTEM, ROTEM, Platelet component

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Correlation of rotational thromboelastometry (ROTEM) parameters with platelet count and their ability to predict thrombocytopenia in dogs☆

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ABSTRACT

The study objectivs were to evaluate the correlation between platelet count (PLT) and rotational thromboelastometry (ROTEM) parameters and to determine ROTEM cut-off values for identification of thrombocytopenia in dogs. Medical records of 113 dogs with concurrent EXTEM (ROTEM activated by proprietary tissue factor), FIBTEM (EXTEM with added cytochalasin D) analysis and PLT were retrospectively reviewed. Signalment, treatment prior to analysis, hematocrit (HCT), EXTEM/FIBTEM maximum clot firmness (MCF_{EXTEM}, MCF_{FIBTEM}), EXTEM/FIBTEM maximum clot elasticity (MCE_{EXTEM}, MCE_{FIBTEM}) and EXTEM maximum lysis (ML_{EXTEM}) were extracted from patient records and ROTEM database. Delta (Δ) MCF was calculated as $MCF_{EXTEM} - MCF_{FIBTEM}$ and ΔMCE as $MCE_{EXTEM} - MCE_{FIBTEM}$. The PLT was correlated to MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE using Spearman-Rho analysis. Correlations were further analyzed in thrombocytopenic dogs. The ability to predict thrombocytopenia was evaluated with receiver operating characteristics (ROC). Thirty-seven samples (32.7%) showed thrombocytopenia ($< 130 \times 10^9$/L) and 19 samples (17%) severe thrombocytopenia ($< 60^* \times 10^9$/L). The PLT significantly correlated with MCF_{EXTEM} ($r = 0.545$, $P < .001$), MCE_{EXTEM} ($r = 0.547$, $P < .001$), ΔMCF ($r = 0.441$, $P < .001$) and ΔMCE ($r = 0.559$, $P < .001$). $MCF_{EXTEM} < 49$ mm, $MCE_{EXTEM} < 93$, ΔMCF < 42 mm and ΔMCE < 90 predicted thrombocytopenia $< 60 \times 10^9$/L with a sensitivity of 90% and a specificity of 78% with a negative predictive value > 97% for all 4 parameters. In conclusion, PLT in dogs correlated moderately but significantly with all evaluated ROTEM parameters. All parameters were able to rule out thrombocytopenia $< 60 \times 10^9$/L with a high negative predictive value, while the sensitivity to predict thrombocytopenia was only moderate and the positive predictive value was low.

1. Introduction

Thromboelastic methods to determine coagulation status are a standard implement in human hospitals and are increasingly becoming available in clinical veterinary practice (Doderlein and Mischke, 2015; Sigrist et al., 2017; Goggs et al., 2018; Muri et al., 2018). Unlike conventional plasma-based coagulation tests, thromboelastic methods are performed in whole blood and give an almost instantaneous and global assessment of a patient's coagulation status from initial platelet-fibrin interactions to fibrinolysis (Solomon et al., 2012; Romlin et al., 2013). The two main thromboelastic devices used are rotational thromboelastometry (ROTEM) and thromboelastography (TEG). Both are point of care viscoelastic hemostasis tests that allow measurement of global clot formation and dissolution in whole blood (Whiting and DiNardo, 2014). With ROTEM, a cylindrical cup containing citrated whole blood and an activator remains fixed while a pin suspended on a ball- bearing mechanism initially oscillates through application of a constant force (Whiting and DiNardo, 2014). As the viscoelastic strength of the clot increases, the rotation of the pin is impeded and is detected optically (Whiting and DiNardo, 2014). Several coagulation activators are available by the manufacturer. The extrinsic activated assay (EXTEM) is activated by proprietary tissue factor whereas the intrinsic activated assay (INTEM) is activated by ellagic acid and phospholipid. The fibrin polymerization test (FIBTEM) is initiated by the same activator as the EXTEM analysis but additionally contains a platelet inhibitor (cytochalasin D) (Haas et al., 2014). The FIBTEM assay indicates the formation and stability of the fibrin clot (Lang et al., 2009).

Through computer analysis clot formation/dissolution kinetics and clot strength data can be generated (Whiting and DiNardo, 2014; Doderlein and Mischke, 2015). The main parameters measured and

☆ This study was conducted at the Vetsuisse Faculty, University of Zurich, Switzerland.

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analyzed are the clotting time (CT) which describes the time until fibrin formation starts. The clot formation time (CFT) depends mainly on fibrinogen concentration and thrombocyte numbers and displays the kinetics of clot formation. The maximum clot firmness (MCF) describes the maximal strength of the fibrin/thrombocyte clot in mm while the maximum clot elasticity (MCE) reflects the force with which the clot resists rotation (Solomon et al., 2015). Fibrinolysis is assessed by maximum lysis (ML) in % within 60 min of measurement.

While it is not possible to determine the platelet count (PLT) by ROTEM analysis, several ROTEM parameters have been evaluated for their correlation with PLT in people (Lang et al., 2009; Herbstreit et al., 2010; Solomon et al., 2011a; Haas et al., 2012; Keene et al., 2013; Lier et al., 2013; Solomon et al., 2015; Honickel and Grottke, 2016).

In people, subtracting FIBTEM MCF (MCF_{FIBTEM}) or MCE (MCE_{FIBTEM}) from EXTEM MCF (MCF_{EXTEM}) or MCE (MCE_{EXTEM}) determines the platelet contribution (ΔMCF and ΔMCE) of the clot, which correlates with PLT (Lang et al., 2009; Solomon et al., 2011a; Dekker et al., 2014; Solomon et al., 2015). When ROTEM analysis is performed for other reasons or as a bedside analyzer, information regarding platelet contribution offers additional information and can guide additional diagnostic tests. In people with severe thrombocytopenia and acute bleeding disorders, the platelet contribution can be used for transfusion guidelines in that the contribution of platelets to clot elasticity determines the need for platelet concentrate transfusion (Solomon et al., 2015).

To our knowledge, ROTEM platelet contribution and its correlation with PLT has only been evaluated in one study of a population of dogs with renal disease (Falco et al., 2013). A moderate but significant correlation was described, but no further details were reported and other ROTEM parameters were not investigated (Falco et al., 2013).

The purpose of this study was to evaluate the correlation of PLT with MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE in both healthy and sick dogs. A second goal was the determination of ROTEM cut-off values for the identification of thrombocytopenia. The hypothesis was that ΔMCF, ΔMCE, and possibly MCF_{EXTEM} and MCE_{EXTEM} alone correlate with PLT and can predict or rule out thrombocytopenia.

2. Materials and methods

The ROTEM database of the Small Animal Clinic, Vetsuisse Faculty of Zurich, was searched for dogs with simultaneous Ex-temS® (Werfen GmbH, Munich, Germany) and Fib-temS® (Werfen GmbH, Munich, Germany) analysis. Dogs were included for further analysis if a concurrent (within 2 h of the ROTEM analysis based on time noted in the database), automatic fluorescence flow cytometry PLT and HCT was available (Sysmex-XT 2000iV, Sysmex Cooperation, Kobe, Japan).

Only the first analysis from each case was included. Parameters obtained from patient records included sex, neutering status, age, weight, breed, medical treatment and transfusions administered prior to ROTEM analysis. Dogs were excluded if the above minimal database was not available.

Blood samples for ROTEM analysis (ROTEM-DeltaWerfen GmbH, Munich, Germany) were analyzed according to an institutional protocol, which is based on manufacturer instructions and international guidelines (deLaforcade et al., 2014; Flatland et al., 2014; Hanel et al., 2014). Briefly, samples were rested at 37 °C for at least 10 min and analyzed within 30 min using an automatic pipette. Tracings were run for 60 min. All tracings were visually controlled for artifacts by one of the authors and suspicious tracings were reviewed by all 3 authors and excluded if an artefact could not be ruled out. Maximum clot elasticity was calculated as 100 x MCF/100 - MCF. An unmeasurable MCF_{FIBTEM} (green line) was defined as 1 mm. Delta MCF and ΔMCE were calculated by subtracting MCF_{FIBTEM} / MCE_{FIBTEM} from MCF_{EXTEM}/MCE_{EXTEM}, respectively. For analysis of subgroups, thrombocytopenia was defined as PLT below the reference interval (130–394 × 10^9/L). Severe thrombocytopenia was defined as a PLT below 60 × 10^9/L.

2.1. Statistical analysis

Descriptive statistical analysis was performed using the statistical software program SPSS (SPSS, version 23, SPSS Inc., Chicago, IL). Normality of continuous data was tested using the Shapiro-Wilk test. Normally distributed data was presented as mean ± SD while not normally distributed data was presented as median and range. Categorical data was described as frequencies. Thrombocytopenia was defined as PLT below the reference interval of the hospital's inhouse laboratory (130–394 × 10^9/L). Correlation of PLT with MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE was analyzed using Spearman-Rho. A receiver operating characteristics (ROC) was performed with Graphpad Prism 7 (Prism 7.0, GraphPad Software Inc., La Jolla, CA) to determine the ability of MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCF to detect thrombocytopenia < 130 and < 60 × 10^9/L. The area under the ROC curve was calculated and values from 0.7 to 0.79 were considered as fair, while values between 0.8 and 0.89 were defined as good results. To find the optimal cut-off value of MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE to detect thrombocytopenia < 130 and < 60 × 10^9/L, the Youden's index was calculated. Significance was set at $P < .05$.

3. Results

Samples of 113 dogs evaluated between 2013 and 2016 were included. The median age of the population was 6.2 years (range, 0.4–13.3 years). Several breeds were identified with mixed breed (n = 21, 16%), Beagle (n = 14, 12%), Golden Retriever (n = 6, 5%) and German Shepherd (n = 4, 4%) being the most common. Fifty-four dogs (48%) were female (33 spayed, 21 intact), 59 (62%) were male (29 spayed, 30 intact). Treatments prior to ROTEM analysis included IV crystalloid fluids in 52/113 dogs (46%), colloids in 15/113 (13.3%), fresh frozen plasma in 12/113 (11%) and packed red blood cells in 4/113 dogs (4%). Eight dogs (7%) had previously been treated with NSAIDs and 16/113 (14%) had received tranexamic acid.

The median PLT was 200 × 10^9/L (range, 3–1304 × 10^9/L). Thirty-seven (33%) dogs were thrombocytopenic (< 130 × 10^9/L) with 19 (17%) showing severe thrombocytopenia (< 60 × 10^9/L), while 66 (58%) dogs had PLT within the reference interval and 10 (8.8%) dogs showed thrombocytosis (> 394 × 10^9/L). Eight dogs (7%) showed hyperfibrinolysis (maximum lysis ≥15%). Characteristics of hematology and ROTEM-S parameters are summarized in Table 1.

Platelet count showed a moderate but significant correlation to MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE (Table 2). The best correlation was obtained with ΔMCE and exclusion of dogs with hyperfibrinolysis (Fig. 1). If only thrombocytopenic (< 130 × 10^9/L) dogs were analyzed, the correlation improved (Table 2). Excluding dogs with hyperfibrinolysis (ML_{EXTEM} > 15%) resulted in a strong correlation while exclusion of dogs that were treated with NSAIDS did not improve the correlation. Scatter-plots of platelet count versus MCF_{EXTEM} and ΔMCF showed a plateau of the curve at higher levels while the correlation of

Table 1
Hematocrit, platelet count, ex-tem S® and fib-tem S® parameters of 113 dogs with concurrent evaluation of ROTEM parameters and platelet count.

Parameter (unit)	Mean ± SD or median	Range	Reference interval
Platelet count (10^9/L)	200	3–1304	130–394
Hematocrit (%)	38 ± 11	11–71	42–55
MCF_{EXTEM} (mm)	53 ± 16	9–95	32–65
MCE_{EXTEM}	122	10–1900	45–142
ML_{EXTEM} (%)	1	0–95	0–12
MCF_{FIBTEM} (mm)	8	1–72	2–9
MCE_{FIBTEM}	8	1–257	2–10
ΔMCF (mm)	47	−5–66	27–50
ΔMCE	113	−6–1643	41–132

MCF, maximum clot firmness; MCE, maximum clot elasticity; ML, maximum lysis; ΔMCF, $MCF_{EXTEM} - MCF_{FIBTEM}$; ΔMCE, $MCE_{EXTEM} - MCE_{FIBTEM}$.

Table 3
AUROC and cut-off values to detect thrombocytopenia < 60 × 10^9/L in 113 dogs.

	AUROC	Sensitivity (%)	95% CI	Specificity (%)	95% CI	Positive predictive value (%)	Negative predictive value (%)
MCF_{EXTEM} ≤ 48 mm	0.889	89.5	66.9–98.7	77.7	67.9–85.6	44.7	97.3
MCE_{EXTEM} ≤ 92	0.888	89.5	66.9–98.7	78.7	69.1–86.5	45.9	97.4
ΔMCF ≤41 mm	0.908	89.5	66.9–98.7	77.7	67.9–85.6	44.7	97.3
ΔMCE < 90	0.904	89.5	66.9–98.7	77.7	67.9–85.6	44.7	97.3

AUROC, area under the receiver operating characteristics curve; CI, confidence interval; MCE, maximum clot elasticity; MCF, maximum clot firmness; ΔMCF, $MCF_{EXTEM} - MCF_{FIBTEM}$; ΔMCE, $MCE_{EXTEM} - MCE_{FIBTEM}$.

Table 4
AUROC and cut-off values to detect thrombocytopenia < 130 × 10^9/L in 113 dogs.

Cut-off	AUROC	Sensitivity (%)	95% CI	Specificity (%)	95% CI	Positive predictive value (%)	Negative predictive value (%)
MCF_{EXTEM} ≤ 51 mm	0.782	70.3	53–84.1	75	63.7–84.2	57.8	83.8
MCE_{EXTEM} < 105	0.784	70.3	53–84.1	76.3	65.2–85.3	59.1	84.1
ΔMCF ≤47 mm	0.734	81.1	64.8–92	56.6	44.7–67.9	45.8	81.5
ΔMCE < 100	0.790	70.3	53–84.1	76.3	65.2–85.3	59.1	84.1

AUROC, area under the receiver operating characteristics curve; CI, confidence interval; MCF, maximum clot firmness; MCE, maximum clot elasticity; ΔMCF, $MCF_{EXTEM} - MCF_{FIBTEM}$; ΔMCE, $MCE_{EXTEM} - MCE_{FIBTEM}$.

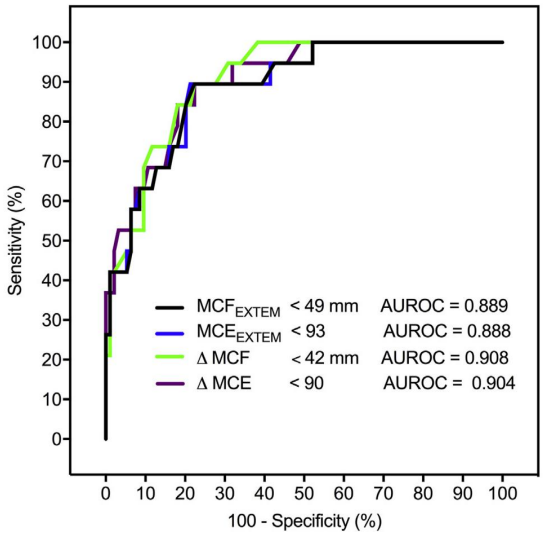

Fig. 2. Cut-off and AUROC of MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE to predict Thrombocytopenia < 60 × 10^9/L in dogs.
AUROC, area under the receiver operating characteristic curve; MCF, maximum clot firmness; MCE, maximum clot elasticity; ΔMCF, $MCF_{EXTEM} - MCF_{FIBTEM}$; ΔMCE, $MCE_{EXTEM} - MCE_{FIBTEM}$.

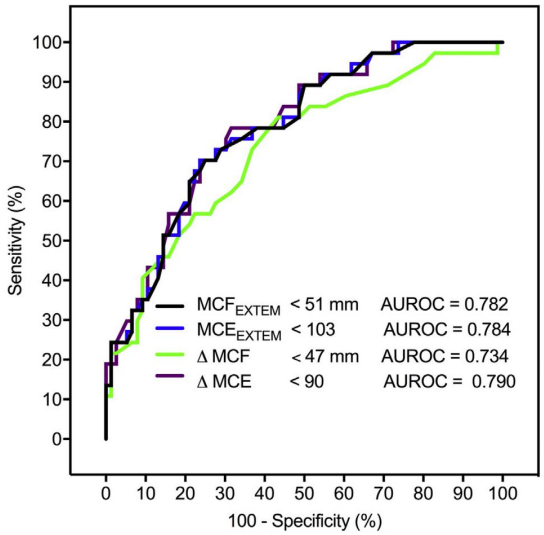

Fig. 3. Cut-off and AUROC of MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE to predict Thrombocytopenia < 130 × 10^9/L in dogs.
AUROC, area under the receiver operating characteristic curve; MCF, maximum clot firmness; MCE, maximum clot elasticity; ΔMCF, $MCF_{EXTEM} - MCF_{FIBTEM}$; ΔMCE, $MCE_{EXTEM} - MCE_{FIBTEM}$.

9–25 mm in people (Lang et al., 2005). In normo- or thrombocytotic dogs, the low "fibrin component" (low MCF_{FIBTEM} and MCE_{FIBTEM}) does not contribute much to MCF_{EXTEM} and MCE_{EXTEM}, leading to similar results of MCF_{EXTEM} and MCE_{EXTEM} and ΔMCF and ΔMCE, respectively. The limited contribution of low MCF_{FIBTEM} to MCF_{EXTEM} has been described in cats (Marly-Voquer et al., 2017) and baboons (Schochl et al., 2012). However, the fibrin component of the clot reflected by MCF_{FIBTEM} and MCE_{FIBTEM} becomes more important with thrombocytopenia and lower MCF_{EXTEM} and MCE_{EXTEM} values, as shown in our subgroups of thrombocytopenic dogs. The correlation index remained moderate and significant using ΔMCF or ΔMCE, while MCF_{EXTEM} and MCE_{EXTEM} did no longer show a significant correlation with lower PLT. The use of ΔMCF and ΔMCE to estimate PLT is therefore expected to be superior to MCF_{EXTEM} or MCE_{EXTEM} in thrombocytopenic dogs. In the current study, ΔMCF and ΔMCE yielded similar correlations at low PLTs. This can be explained with the linear relationship of platelets with ΔMCF in the lower range of the curve (Fig. 1).

Other reasons may account for the only moderate correlation between PLT and ROTEM parameters. Maximum clot firmness and elasticity quantify the function of fibrin, factor XIII and platelets. The firmness/elasticity of the clot is not only dependent on the number, but also on the function of the platelets. Although ROTEM is not sensitive to detect selective platelet inhibitors (Honickel and Grottke, 2016), we cannot exclude an effect on the relationship between PLT and evaluated ROTEM parameters as platelet function was not quantified in the current study. Treatment with NSAIDS may decrease MCF_{EXTEM} (Brainard et al., 2007) while PLT should not be affected, however, exclusion of dogs treated with NSAIDS did not lead to a stronger correlation and we therefore conclude that this effect is minimal.

Exclusion of dogs with hyperfibrinolysis lead to a strong correlation.

Table 2
Correlation of platelet count with MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE in 113 dogs and in subgroups.

Correlation of platelet count with	n	Spearman *r*	95% confidence interval	*P*-value (two-tailed)
All dogs				
MCF_{EXTEM}	113	0.545	0.396 to 0.666	< 0.0001
MCE_{EXTEM}	113	0.547	0.399 to 0.668	< 0.0001
ΔMCF	113	0.441	0.274 to 0.582	< 0.0001
ΔMCE	113	0.559	0.413 to 0.677	< 0.0001
Thrombocytopenic dogs (platelet count < 130 × 10^9/L)				
MCF_{EXTEM}	37	0.585	0.313 to 0.768	< 0.0001
MCE_{EXTEM}	37	0.589	0.318 to 0.77	< 0.0001
ΔMCF	37	0.682	0.451to 0.82	< 0.0001
ΔMCE	37	0.649	0.40 to 0.807	< 0.0001
Severely thrombocytopenic dogs (platelet count < 60 × 10^9/L)				
MCF_{EXTEM}	19	0.272	−0.222 to 0.654	0.261
MCE_{EXTEM}	19	0.282	−0.211 to 0.661	0.242
ΔMCF	19	0.510	0.0586 to 0.789	0.026
ΔMCE	19	0.488	0.0289 to 0.777	0.034
Exclusion of dogs with hyperfibrinolysis				
MCF_{EXTEM}	105	0.602	0.4588 to 0.7141	< 0.001
MCE_{EXTEM}	105	0.605	0.4626 to 0.7164	< 0.001
ΔMCF	105	0.484	0.3171 to 0.6219	< 0.001
ΔMCE	105	0.620	0.4813 to 0.7279	< 0.001
Exclusion of dogs with NSAID therapy				
MCF_{EXTEM}	105	0.580	0.432 to 0.697	< 0.001
MCE_{EXTEM}	105	0.583	0.436 to 0.700	< 0.001
ΔMCF	105	0.472	0.303 to 0.612	< 0.001
ΔMCE	105	0.593	0.448 to 0.707	< 0.001

MCF, maximum clot firmness; MCE, maximum clot elasticity; ΔMCF, $MCF_{EXTEM} - MCF_{FIBTEM}$; ΔMCE, $MCE_{EXTEM} - MCE_{FIBTEM}$.

platelet count to MCE and ΔMCE was linear (Fig. 1).

The area under the receiver operating curve (AUROC), cut-off values, their sensitivity, specificity, positive and negative predictive values to detect severe thrombocytopenia and thrombocytopenia are summarized in Tables 3 and 4, respectively. MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE had a large and similar AUROC to detect severe thrombocytopenia < 60 × 10^9/L with a negative predictive value > 97% for the best cut-off values in all 4 parameters (Table 3 and Fig. 2). The ability of ROTEM parameters to detect thrombocytopenia < 130 × 10^9/L was lower (Table 4 and Fig. 3).

4. Discussion

Our study results showed a moderate but significant correlation between PLT and all analyzed ROTEM parameters in dogs. All ROTEM parameters were able to exclude severe thrombocytopenia with a high negative predictive value.

A positive correlation of MCF_{EXTEM} with PLT has been described in various studies in people includingchildren (Herbstreit et al., 2010; Haas et al., 2012; Keene et al., 2013; Lier et al., 2013; Honickel and Grottke, 2016) and was also identified in our canine population. As described in people (Solomon et al., 2015), we also report a nonlinear relationship between MCF and PLT in dogs; with MCF showing a plateau effect at high PLTs (Fig. 1). Maximum clot elasticity reflects the force with which the clot resists rotation within the device and MCE increases linearly with increasing PLT (Solomon et al., 2015). Maximum clot elasticity is therefore thought to better reflect PLT (Olde Engberink et al., 2014).

As both platelets and fibrin contribute to MCF_{EXTEM}/MCE_{EXTEM}, the contribution of platelets (platelet component) to clot strength can be determined by subtracting MCF_{FIBTEM} or MCE_{FIBTEM} from MCF_{EXTEM} or MCE_{EXTEM}, respectively. The platelet components of MCF and MCE correlate even better to PLT in people (Lang et al., 2009; Solomon et al., 2011a,b, 2015). Again, ΔMCE is thought to be superior to ΔMCF as the relationship between clot firmness and PLT is nonlinear (Solomon et al., 2015). Our results in dogs do not indicate that the platelet component ΔMCF or ΔMCE correlates stronger with PLT than MCF_{EXTEM} or MCE_{EXTEM} alone. This may be explained by the lower reference intervals of MCF_{FIBTEM} in dogs compared to people (MCF_{FIBTEM} 3–9 mm vs

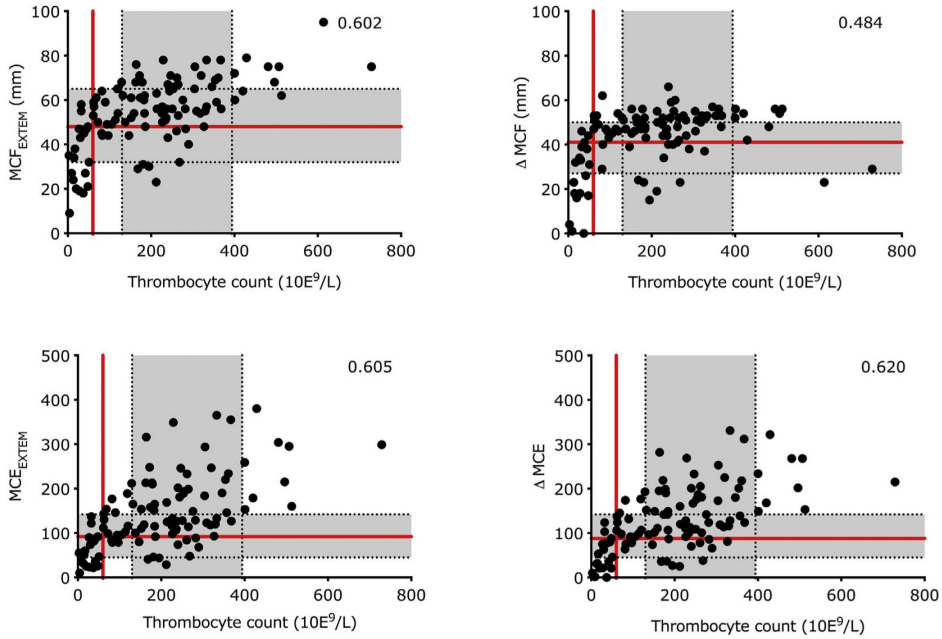

Fig. 1. Scatter plot of platelet count versus MCF_{EXTEM}, MCE_{EXTEM}, ΔMCF and ΔMCE in 105 dogs without hyperfibrinolysis. The graph was truncated at a platelet count of 800 × 10^9/L and MCE/ΔMCE 500. Shaded areas represent reference intervals. MCF, maximum clot firmness; MCE, maximum clot elasticity; ΔMCF, $MCF_{EXTEM} - MCF_{FIBTEM}$; ΔMCE, $MCE_{EXTEM} - MCE_{FIBTEM}$.

This is expected as hyperfibrinolysis could lead to a decrease in MCF_{EXTEM} and MCE_{EXTEM} due to ongoing clot lysis and therefore also to a lower ΔMCF and ΔMCE (Levrat et al., 2008; Sigrist et al., 2018).

The FIBTEM assay contains cytochalasin D that is expected to inhibit any interaction of fibrinogen with platelets, however, a residual platelet effect on MCF_{FIBTEM} has been described and becomes more important the more hypofibrinogenemic a patient is (Lang et al., 2009). Additionally, the HCT of a blood sample influences clot firmness and elasticity with anemic dogs having a hypercoagulable EXTEM or FIBTEM tracing due to a higher concentration of coagulation factors (Smith et al., 2012).

Of note, our institutional reference intervals for MCF_{EXTEM} and MCE_{EXTEM} are lower than those for MCF_{INTEM} and MCE_{INTEM}. In people (Lang et al., 2005) and cats (Marly-Voquer et al., 2017), the reference interval of those parameters is very similar. As MCF_{INTEM} / MCE_{INTEM} are higher than MCF_{EXTEM} / MCE_{EXTEM}, they may better reflect PLT. Our database did not contain enough INTEM data to be able to address this question and further studies are needed to investigate possible better parameters such as MCF_{INTEM} / MCE_{INTEM} to estimate PLT in dogs. With the current results we recommend using ΔMCE and exclude samples with hyperfibrinolysis, as this showed a strong correlation over various PLT.

The AUROC of all evaluated ROTEM parameters to detect severe thrombocytopenia $< 60 \times 10^9$/L was high and ΔMCF or ΔMCE were not superior to MCF_{EXTEM} or MCE_{EXTEM}. While all evaluated cut-off values showed a good sensitivity, the specificity to detect severe thrombocytopenia $< 60 \times 10^9$/L was only moderate. The low positive predictive value and the fact that all cut-off values are in the middle to upper range of the reference interval limits the clinical utility to predict thrombocytopenia. However, all 4 evaluated parameters had a high negative predictive value of 97% and the reported cut-off values can be used to rule out thrombocytopenia $< 60 \times 10^9$/L. Dogs with values lower than the cut-off values should undergo further testing as accurate PLT are clinically relevant in cases with suspected low numbers. While PLTs can easily be estimated by manual evaluation of a blood smear in humans, the platelet estimation in canine blood smears can vary significantly (Paltrinieri et al., 2018). Determination of PLT is therefore indicated but a reliable hematology analyzer may not be available in an emergency situation and may take longer. Using a point-of-care device such as ROTEM can - if available – rule out clinically significant thrombocytopenia until absolute PLT is available.

The main advantage performing ROTEM analysis rather than manual platelet count is concurrent diagnosis of other hemostatic abnormalities such as hypofibrinogenemia, clotting factor deficiency or hyperfibrinolysis. ROTEM analysis further allows the identification of clot strength in thrombocytopenic dogs, eg a strong FIBTEM may lead to normal clot formation despite low platelet count and therefore not require treatment with platelet transfusion. Platelet count alone does not always correlate with bleeding symptomatology and may be a worse predictor of bleeding than viscoelastic tests in dogs and people (O'Marra et al., 2012; Bucknoff et al., 2014; Greene et al., 2014). In the current retrospective study, evaluation of PLT and ROTEM parameters to predict bleeding was not performed.

4.1. Limitations

Being a retrospective study, several limitations need to be discussed. First, the study population is heterogenous as any dog with concurrent determination of ROTEM and PLT was eligible to enter the study and the number was too small to evaluate subgroups with different hemostatic disorders. Additionally, the study population included only 19 patients with a very low PLT and the results to identify thrombocytopenia $< 60 \times 10^9$/L should be interpreted with caution. Despite the low number of thrombocytopenic dogs, our results show that the evaluated ROTEM parameters can be used to *rule out* severe thrombocytopenia. Third, a time difference between tests of 120 min was allowed due to different locations (hospital laboratory for PLT and on-site performance of ROTEM analysis). However, every effort was made to choose only concurrent blood samples and samples were excluded if patient records suggested that two different blood samples were analyzed within this time frame. Additionally, the storage time of blood samples may have influenced results as prolonged storage time can have a profound impact on ROTEM results depending on the activator with a shift towards hypercoagulability (Smith et al., 2010). However, modern activated tests as used in the current study are less dependent on storing time than native samples (Smith et al., 2010). As 88% of samples were analyzed within 60 min, we further do not think that time significantly influenced results. Handling and storage time of each sample may have varied due to the retrospective nature of the study, but our ROTEM assays should be performed following institutional guidelines based on manufacturer instructions and international guidelines (deLaforcade et al., 2014; Flatland et al., 2014; Hanel et al., 2014). Additionally, small differences in handling and storage time mimic the clinical situation and were therefore accepted. Artifacts due to inappropriate test performance was excluded by visual evaluation of all curves. Colloid solutions administered in 13 dogs may have influenced the results due to weakening of the MCF_{FIBTEM}. However, the platelet component should not have been affected (Weiss et al., 2010; Hartog et al., 2011; Falco et al., 2012; Wurlod et al., 2015) and the study sample size was considered too small to analyze each subgroup separately.

5. Conclusions

Platelet count in dogs correlated moderately but significantly with all evaluated ROTEM parameters. The correlations using ΔMCF and ΔMCE were superior to MCF_{EXTEM} and MCE_{EXTEM} in thrombocytopenic dogs and the correlation became strong if dogs with hyperfibrinolysis were excluded. All parameters were able to rule out thrombocytopenia $< 60 \times 10^9$/L with a high negative predictive value while the positive predictive value was low.

Declaration of Competing Interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors and the authors declare no conflict of interest.

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